REMARKS

Rejection of claims under 35 U.S.C. 112:

Claims 1-4, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36, 39 and 40 have been rejected under 35 U.S.C. §112.

The office action states, on page 3, that no support could be found for the phrase "an injector" in the specification as stated by the Applicants. Applicants respectfully disagree. Page 2 lines 28-29, page 3 lines 2-3 and 14, page 4 line 32, page 5 line 5-7, page 10 line 20, page 16 lines 10-16, and page 17 lines 8-31 of the specification all state that the polynucleotide is to be "inserted" or "injected" into a vessel. For a polynucleotide to be inserted or injected into said vessel, some device must be utilized, i.e. an injector. On page 23 lines 16-23 and page 25 line 32 bridging to page 26 line 1 of the specification, Applicants describe the use of a catheter as the device, or injector, with which to inject the polynucleotide. On page 31 lines 9-12 of the specification, Applicants describe the use of a syringe needle as the device, or injector, with which to inject the polynucleotide. Thus, it is the Applicants' opinion that the term injector is sufficiently defined such it is readily identified by those knowledgeable in the art.

The office action states, on page 3, that no support could be found for the phrase "distal to the occlusion" in the specification as stated by the Applicants. Applicants respectfully disagree. Page 23 lines 22-25 of the specification clearly states that a sphygmomanometer cuff is placed surrounding the arm or leg proximal to the injection site. If the occlusion (sphygmomanometer cuff) is proximal to the injection site, the injection site must be distal to the occlusion. Example 10 and page 32 further describes vessel occlusion, by a tourniquet, proximal to the injection site and target tissue (see especially lines 12-13 and lines 18-19).

The office action states, on page 3, that the terms "superficialis" and "profundis" constitute new matter. Applicants respectfully disagree. As stated in the amendment filed by the Applicants on 8-11-03, the abbreviations for the terms "superficialis" and "profundis", "spf." and "prof" respectively, can be found in the specification on page 26. The abbreviations were cited in the claims as originally filed. However, the Examiner stated in an Office Action dated July 30, 2002 that the abbreviated terms were ambiguous. Therefore Applicants amended the claims to cite the unabbreviated terms at the request of the Examiner.

The office action states, on page 4, that Miller 1995, Deonarain 1998, Verma 1997, and Crystal 1995 teach that vector targeting to desired tissues *in vivo* continues to be unpredictable in the art. However, none of these publications contemplates the process taught by the Applicants in the instant application. It is the Applicants' opinion that the instant application should be judged on its merits rather than on limitations associated with other delivery systems. Applicants believe they have clearly demonstrated functional delivery of polynucleotides to limb skeletal muscles as evidenced by the data presented in the examples of the instant application.

With respect to the teaching of Milas et al. 1997 (page 4 of the office action), it is the Applicants' opinion that Milas did not teach: administering particles encoding LacZ into a femoral artery and vein occluded using a tourniquet and getting expression in hepatocytes but not muscle cells of the limb. Rather, Milas taught that isolated limb perfusion, as a process, was inadequate for delivering adenovirus to limb skeletal muscle cells. Milas further taught that a tourniquet placed around a leg fails to prevent adenovirus infection of liver cells following perfusion of the leg. In other words, Milas shows that perfusion of adenovirus in an isolated limb fails to provide a mechanism for infection of muscle cells by adenovirus. In contrast, Applicants have clearly demonstrated their ability to deliver functional nucleic acid to skeletal muscle cells using the process they describe.

The office action states on page 5 that the specification does not teach delivering DNA to an arm blood vessel and expressing the DNA in leg skeletal muscle. Applicants agree and believe that clear continuity is provided in the claims for delivery of polynucleotides to muscle cells in a limb by occluding vessels of said limb and inserting the polynucleotides into a vessel of said limb. In the Applicants opinion it is not reasonable to interpret the claim to provide for injecting a polynucleotide into a vessel of the leg and delivering the polynucleotide to a cell in an arm.

The office action states, on page 5, that it can not be determined how targeting a cell as broadly claims is affected by the location of the blood vessel injected, the type of polynucleotide, or the method of occlusion. Applicants respectfully disagree. The location of the vessel is clearly cited to be in the limb of the mammal. The point of the injection is cited in the claims to be distal to the occlusion (see also page 23 lines 22-25 and page 32 lines 12-

13 in the specification). The specification also teaches, on page 32 lines 18-19, that the polynucleotides are delivered to "muscle groups that were located distal to the tourniquet" or vessel occlusion. The specification also teaches that vessels in the limb are occluded by an external device such as a tourniquet or sphygmomanometer (page 5 lines 13-24).

The office action states, on page 5, that it can not be determined how targeting a cell as broadly claims is affected by "the method of immunosuppressing (administering vs. not administering an immunosuppressive agent)." Applicants are unable to respond to the rejection because there is no claim of immunosuppression made in the instant application.

The office action argues that only delivery of a marker gene operably linked to a promoter is enabled and that delivery of other polynucleotides is not enabled. The office action further argues that the polynucleotide must encode a protein that is expressed to detectable levels to constitute an enabled use. Both of these statements contradict accepted principles in the art. First, the delivery of a polynucleotide to a cell is not dependent upon the specific sequence of the polynucleotide. If it were, a new delivery system (transfection reagent) would have to be developed for every plasmid encoding a different gene. Second, while the specific transcriptional promoter used is fully expected to effect gene expression in the cell, different promoters are not expected to effect the delivery of the polynucleotide to the cell. It is also expected that a given promoter will work with nearly any gene to which it is operably linked. This principle is evidenced by the number of expression plasmids available from numerous biotechnology companies. These expression plasmids contain a promoter and a means by which to insert any nucleotide sequence of interest downstream of the promoter. The promoter is then expected to drive transcription of the sequence. Finally, there exists an extensive body of literature in the fields of antisense nucleic acid, ribozymes, and RNA interference. Antisense nucleic acids, ribozymes and double stranded RNAs (which mediates RNA interference) are polynucleotides that inhibit gene expression. These polynucleotides do not encode proteins and need not be expressed to function. Instead these polynucleotides directly interfere with some aspect of gene expression such as causing mRNA degradation. Inhibition of gene expression is a use for which a polynucleotide can be delivered to a cell. This use does not require expression of a protein.

On page 6 of the Office Action, the Examiner states that the claim "does not require the blood vessel is part of the mammal" and that "the claim does not state the injector is inserted into a

mammal or that the injector is inserted into a limb blood vessel of the mammal *in vivo*." Applicants believe the claims clear on this issue. The claim states that the process is an "*in vivo* process" and part a) directly cites, "inserting an injector into a limb blood vessel of the mammal." It is the Applicants' opinion that the claim can not be reasonably interpreted to mean injecting a polynucleotide into a vessel that has been removed from the mammal. However, Applicants have amended claim 1 to cite "a limb blood vessel in the mammal."

The office action also states that the claims are unclear because: 1) the claims do not require the blood flow to be impeded within the mammal, 2) the claims do not require that the mammalian skin belong to the mammal into which an injector is inserted, 3) the claims do not clearly set forth that the limb being occluded is the limb into which the injector is inserted, and 4) the claims appear to indicate that the device is applied anywhere outside of mammalian skin. Applicants respectfully disagree. First, steps b) and c) of claim 1 cite that a device is applied for occluding blood vessels (b) and that the polynucleotide is injected into the vessel distal to the occlusion (c). Because the polynucleotide is injected distal to the occlusion, the occlusion must have occurred. Second, Applicants do not believe that it is reasonable to interpret the claim to mean that the mammalian skin can belong to a mammal other than the mammal into which the injector is inserted. Nevertheless, Applicants have amended claim 1 to cite "to the mammal's skin" rather than "to mammalian skin." Third, because the solution containing the polynucleotides is injected "distal to the occlusion", the limb that is injected must be the limb in which the vessel is occluded. Finally, step b) cites that the external device occludes blood vessels in the limb. The specification teaches (page 5 lines 13-24) that the external device is "applied exterior to the mammal's skin and touches the skin in a non-invasive manner" and that the device "applies external pressure to the mammalian skin" thereby forcing vessel walls "to constrict in an area underneath the cuff in amount sufficient to impede blood from flowing at a normal rate." Because the device must occlude vessels in the limb, it can not simply be placed "anywhere outside of the mammalian skin." Applicants have amended the claim to more clearly cite that the device occludes vessels in the limb.

The office action states on page 7 that claims 8, 9, 12, 13, 14, 17, 21, 22, 24, 25, 26, and 29 are rejected because the terms anterior, posterior and superficial are indefinite. Applicants respectfully disagree. The terms anterior, posterior and superficial are recognized terms in the art for identifying muscle groups as evidenced by anatomy texts such as *Gray's Anatomy*. For

instance, the palmaris longus muscle is by definition an anterior superficial muscle. The palmaris longus muscle is an anterior superficial muscle because of its location in the mammal and not by the perspective of one viewing the mammal.

The office action states on page 7 that claims 34-36 remains indefinite because a tourniquet or cuff is not applied over the skin, but on an arm, leg, etc. Applicants have specified that blood vessels in the limb are occluded using an external device. In order for an external device to occlude vessels in a limb, it must exert pressure against the limb and compress the limb. In order to compress the limb, the external device must touch the skin, i.e. be applied over the skin. The specification teaches (page 5 lines 13-24) that the external device is "applied exterior to the mammal's skin and touches the skin in a non-invasive manner" and that the device "applies external pressure to the mammalian skin" thereby forcing vessel walls "to constrict in an area underneath the cuff in amount sufficient to impede blood from flowing at a normal rate."

The examiner questions, "Is pinching the skin encompassed by the claim?" Since the claims and the specification cite that the skin is compressed for the purpose of occluding vessels in a limb, simply pinching the skin is clearly not encompassed by the claim unless the pinching occludes blow flow through a vessel. The examiner also asks, "does the cuff have to be applied to the outside of the mammal or is a string around the blood vessel a cuff?" Applicants are clear in stating that the cuff is an external device. Since all vessels are internal, a string around a vessel is not encompassed by the claim.

Claim 39 has also been rejected because of the indefiniteness of step 3. Applicants have amended claim 39 to obviate the rejection.

Rejection of claims under 35 U.S.C. 102:

Claims 1-4, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36, 39 and 40 have been rejected under 35 U.S.C. §102 (b) as being anticipated by Milas et al 1997. Milas taught a method for perfusing adenovirus through isolated hind limb vasculature in a rat soft tissue sarcoma model. The method taught by Milas consists of: placement of a tourniquet around the leg and underneath the inguinal ligament of the hind limb (an invasive placement), perfusion of the limb (via catheter insertion in both the femoral artery and vein) with normal saline followed by perfusion of the limb with adenovirus-containing solution and perfusion of the limb with

normal saline again, and ligation of the femoral artery and vein distal to the cannulation sites. The method taught by Milas relied on collateral circulation to preserve limb viability following femoral artery ligation. Milas et al state on page 2202, paragraph 1 that "cannulation of the femoral vein with resultant brisk outflow is critical for the success of the procedure... ." Milas shows delivery of the adenovirus to tumor cells and expression of virally encoded β-galactosidase. Tumors are known in the art to have a leaky local vasculature. It is likely that this leaky vasculature of the tumor allowed access of the virus to the sarcoma. Milas observed no expression of β-galactosidase expression in skeletal muscle. In contrast, Applicants teach injection of polynucleotide into a single blood vessel and a non-invasive cuff without ligation of the vessel after the procedure. A single catheter or needle is required, rather that two. No perfusion of the limb is required. No ligation of the femoral artery or vein is required. Using the described invention, Applicants observe polynucleotide delivery to skeletal muscle cells throughout the limb. The method taught by Milas results in delivery of adenovirus to sarcoma tumors but not to skeletal muscle cells. The Applicants believe that the claimed invention does not encompass the methods taught by Milas.

Double Patenting Rejection

Applicants request at this time to defer any response to the double patenting rejection until a claim is in condition for allowance.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-4, 6-9, 11-14, 16-22, 24-26, 28-31, 33-36 and 39-40 should be allowable.

Respectfully submitted,

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I hereby certify that this correspondence is being facsimile transmitted to the USPTO 703.872.9306 addressed to: Commissioner for Patents, Alexandria, VA, 22313 on: August 11, 2003.

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